

Evaluation of two cowside tests for the detection of subclinical ketosis in dairy cows

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Abstract

The goal of this study was to evaluate the sensitivity and specificity of two cowside tests for subclinical ketosis in dairy cows. The tests utilize milk and urine samples, respectively. One hundred and eighty-five cows, one to sixty days postpartum, were sampled for milk, urine, and blood. Subclinical ketosis was defined with serum β -hydroxybutyrate measurements.

The sensitivity and the specificity of both tests at different β -hydroxybutyrate levels were estimated. When subclinical ketosis was defined at β -hydroxybutyrate levels of 1.4 mmol/L and higher, the milk test had sensitivity of 90% and specificity of 96%. The urine test lacked specificity (values <67%), but sensitivity was 100% at β -hydroxybutyrate levels of 1.4 mmol/L upward.

Both the milk and urine test can be used to monitor subclinical ketosis in a herd. Milk testing is preferred, because of the easy obtainability of milk combined with the overall better test characteristics.

Résumé

Évaluation de deux tests diagnostiques rapides pour déceler l'acétonémie subclinique chez les vaches laitières

Le but de cette étude était d'évaluer la performance de deux tests diagnostiques rapides pour déceler l'acétonémie subclinique chez les vaches laitières. Les épreuves ont été effectuées sur des échantillons de lait ou d'urine selon le test utilisé. Des prélèvements de lait, d'urine et de sang ont été effectués sur 185 vaches, de 1 à 60 jours postpartum. L'acétonémie subclinique a été déterminée en quantifiant le β -hydroxybutyrate sérique. La sensibilité et la spécificité des deux tests ont été évalués pour différents taux de β -hydroxybutyrate. Lorsque l'acétonémie subclinique était déterminée par un taux de β -hydroxybutyrate égal ou supérieur à 1,4 mmol/L, le test diagnostique sur le lait était sensible à 90 % et spécifique à 96 %, alors que le test diagnostique sur l'urine était moins spécifique (< 67 %), mais sensible à 100 %. Les auteurs concluent que les deux tests, soit sur le lait ou soit sur l'urine, peuvent être utilisés pour déceler l'acétonémie subclinique dans un troupeau. Le dépistage sur le lait est préféré, car les échantillons peuvent s'obtenir facilement et que le rendement général du test est meilleur.

(Traduit par Dr Thérèse Lanthier)

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Introduction

Ketosis in dairy cows is defined as an increase in ketone bodies in all body tissues and fluids (1). Important ketone bodies are β -hydroxybutyrate, acetone, and acetoacetate. The presence of excess ketone bodies without clinical signs is defined as subclinical ketosis. Clinical and subclinical ketosis are both caused by a negative energy balance in the cow (2).

The prevalence of subclinical ketosis varies depending on herd factors, days in milk, breed, parity, and season (3). The prevalence of subclinical ketosis has been reported between 2% and 15% in different studies (4,5). The prevalence was higher in the first compared to the second month of lactation (1), with the peak prevalence during the fourth week (5). Subclinical ketosis has been associated with lower milk production (4,6) and an increase in the average calving interval (7,8). Ketotic cows were also at greater risk of developing cystic ovarian disease and mastitis (4,5).

In general, diagnosis of subclinical diseases is important to optimize herd management to prevent outbreaks of clinical disease. It may therefore be of value to be able to monitor the energy balance in the herd. Feeding management could be adapted when the apparent prevalence of subclinical ketosis increases in the herd, or prophylactic treatment could be started (9–12).

Subclinical ketosis can be monitored using blood, urine, or milk samples as a source of data (3). The cowside tests for (sub)clinical ketosis are based on a color reaction of ketone bodies with sodium nitroprusside (10). Cowside tests can be performed using either milk, urine, or blood. They give rapid results and can be used as frequently as deemed necessary. Day to day feeding management of susceptible cows could be changed instantly based on the cowside test results. However, to be able to interpret test results on a cow and herd basis, sensitivity and specificity of a test should be known (13).

Our objective was to evaluate the usefulness of two cowside tests, using milk and urine samples, to detect subclinical ketosis in dairy cows in early lactation.

Materials and methods

One hundred and eighty-five cows from 18 herds in the vicinity of Utrecht, The Netherlands, were included in the study. All but two of the herds participated in a computerized veterinary herd health program, and all of the herds were enrolled in a production measurement program. The production of these 18 herds was above the Dutch average of 7204 kg, with 4.42% fat and 3.44% protein in 1990. On the day of the herd visit, all cows from days 1 to 60 postpartum that were healthy, according to the farmers, were sampled. Sampling was carried out during the winter housing period from March through April 1990. The distribution of the sample population by parity and by weeks in milk is presented in Figure 1.

Blood samples taken from the coccygeal vein were transported on ice to the laboratory. Samples were

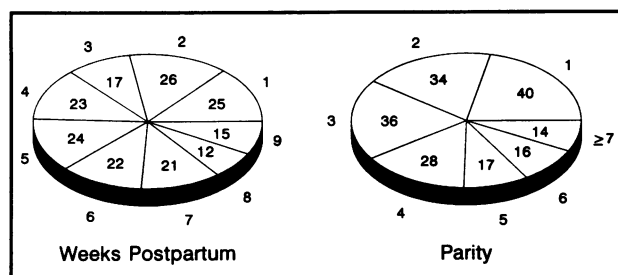


Figure 1. Stage of lactation and parity of 185 cows.

centrifuged and the serum was frozen at -20°C . After thawing, β -hydroxybutyrate (BHB) was measured enzymatically, using 3-hydroxybutyrate dehydrogenase (Synchron CX5, Beckman, Los Angeles, California, USA). The BHB concentrations were used as the reference method to define subclinical ketosis (gold standard) (10,14–16).

Milk from one quarter per cow was used to determine milk ketone levels. The milk samples were tested on the farm by adding a few drops of milk to sodium nitroprusside powder in a round well of a white porcelain tray with 12 wells (10).

Only spontaneous free flow urine samples were collected from 124 cows. The urine samples were tested on the farm by adding a drop of urine to a tablet (Acetest, Ames Division, Miles Laboratories Ltd, Bridgend, Glamorgan, UK) that had been placed in a round porcelain well.

The milk and urine cowside tests were done in duplicate, and scored after a two minute reaction time. The scores were 0, 1, or 2 for no discoloration, slight brownish-purple discoloration, or clear purple discoloration, respectively. A positive test result was defined as a score of 1 or 2 in one or both of the duplicate samples. The blood, milk, and urine samples were taken from each cow within thirty minutes.

Data were analyzed on a personal computer (Statistix version 3.5, Analytical Software, St. Paul, Minnesota, USA). Subclinical ketosis was defined using the serum BHB concentration from the blood samples taken at the same time as the milk and urine samples (10,14–16). Several cut-off points of ketone body levels in blood or milk have been suggested for subclinical ketosis (3,15–18). We determined the BHB threshold based on the distribution of the BHB concentrations in the study population, assuming that in a healthy cow population a normally distributed BHB concentration would be found. Sensitivity and specificity were determined for

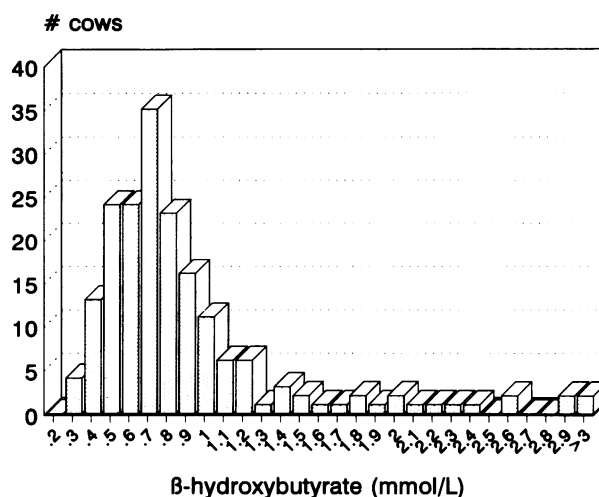


Figure 2. Distribution of β -hydroxybutyrate blood levels in 185 cows. Values on the X-axis represent midpoint values.

each urine and milk cowside test (13). Sensitivity was defined as the proportion of subclinical ketotic cows that was test positive. Specificity was the proportion of the nonketotic cows that was test negative. Agreement between milk and urine tests was evaluated using the kappa statistic (13).

Results

The distribution of the BHB concentrations in the blood of cows is shown in Figure 2. The mean BHB concentration was 0.88 mmol/L, and the median BHB was 0.72 mmol/L, with a minimum of 0.27 mmol/L and a maximum of 3.90 mmol/L. Because the distribution of our data was skewed around 1.2 mmol/L BHB, we defined the threshold for subclinical disease at that level. This resulted in a prevalence of subclinical ketosis of 14%, which coincides with other reports (3).

Twenty-three of the 185 cows (12.4%) had a positive milk ketone test. The urine ketone test was positive in 59 of the 124 cows that were sampled (47.6%). In the milk ketone test, one duplicate sample, and in the urine ketone test, five duplicate samples did not agree on the outcome.

The sensitivity and specificity of the milk and urine tests at different BHB cut-off values are shown in Tables 1 and 2, respectively.

The blood BHB values of cows with a positive or a negative milk test are shown in Figure 3. In Figure 4, the

Table 1. Sensitivity and specificity of the milk test with different threshold levels of β -hydroxybutyrate concentrations (mmol/L), using 185 cows. If the concentration is equal to or greater than a threshold, the cow is defined as having subclinical ketosis

β -hydroxybutyrate threshold (mmol/L)	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5
N	101	76	54	41	32	26	23	19	19
milk sens. (%)	22	28	39	49	63	73	78	90	90
spec. (%)	99	98	98	98	98	98	97	96	96

N = number of cows above threshold

Table 2. Sensitivity and specificity of the urine test with different threshold levels of β -hydroxybutyrate concentrations (mmol/L), using 124 cows. If the concentration is equal to or greater than a threshold, the cow is defined as having subclinical ketosis

β -hydroxybutyrate threshold (mmol/L)	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5
N	75	56	39	33	27	21	18	14	14
urine sens. (%)	65	77	85	88	85	91	94	100	100
spec. (%)	80	77	69	67	63	61	60	59	59

N = number of cows above threshold

blood BHB values in relation to a positive or negative urine test are shown. Only 16 of the 59 urine positive tests were also milk positive. Sixty-three of the 65 urine negative tests were also milk negative. The calculated kappa value was 0.25.

Discussion

Borderline values to separate healthy cows from cows with subclinical ketosis have been reported in different studies to vary between approximately 0.7 mmol/L and 1.5 mmol/L BHB in blood (3). We therefore calculated test characteristics for that range of BHB thresholds (Tables 1 and 2). For the milk test, a borderline value of 1.4 mmol/L gave the best test results, relative to the gold standard.

The diurnal variation in BHB, which is related to feeding (14,17), was not considered a problem in our study, because we sampled each cow for blood, milk, and urine within a short time interval. Consequently, post-feeding high levels of ketone bodies would be found in all fluids, causing no false conclusions for the test characteristics (10,14). In addition, 1.2 mmol/L BHB coincides with maximum postfeeding levels for healthy cows (17, unpublished data), and is below the minimal value of 1.8 mmol/L of fluctuations in subclinical ketotic cows (14).

Since all test comparisons were performed within cows, herd, parity and days in milk should not affect the observed test characteristics.

Testing with the three different tests resulted in different prevalences of subclinical ketosis. Assuming that serum BHB concentration is the correct measurement, the urine test resulted in a very high apparent prevalence, while the milk test did not result in a very different apparent prevalence.

Agreement beyond chance between the milk and urine tests was low ($Kappa = 0.25$) because of the high disagreement in the urine positive samples, which were likely false positives.

For management purposes, the milk test could be used to screen a herd for cows with BHB levels over 1.4 mmol/L. Depending on the prevalence of subclinical ketosis in a herd, not many false negatives would occur because of its relatively high sensitivity (90%). Its good specificity (96%) would assure few false positives. At the same cut-off level, the urine test will show no false negatives due to a sensitivity of 100%. However, because of the very low specificity of the test, many false positives and an apparently very high prevalence will

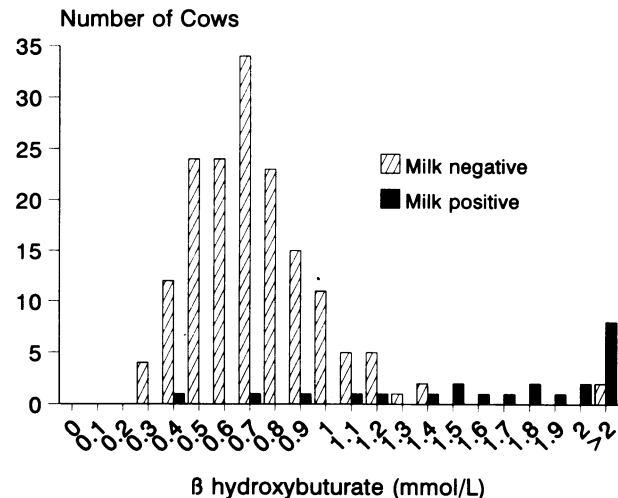


Figure 3. The β -hydroxybutyrate concentration in serum of 185 cows with a positive or a negative milk test. Values on the X-axis represent midpoint values.

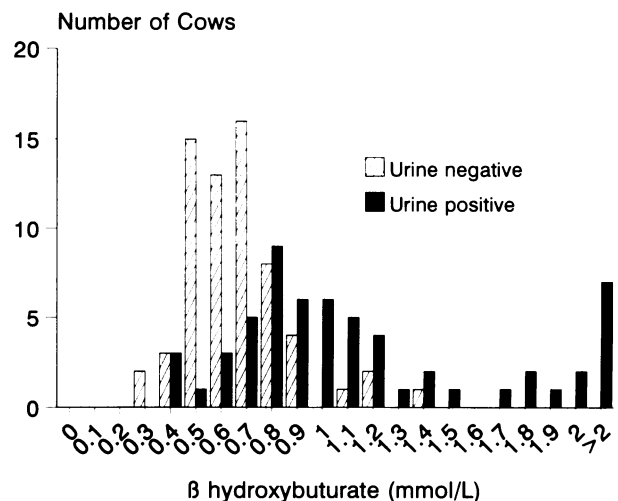


Figure 4. The β -hydroxybutyrate concentration in serum of 124 cows with a positive or a negative urine test. Values on the X-axis represent midpoint values.

result. This could cause undue concern, when the test is used incidentally.

In contrast to serum BHB sampling and testing, both cowside tests could be incorporated in the daily management of a herd, as the herdsman could perform both tests. The tests could be used routinely for the group of cows that are at risk for subclinical ketosis (weeks 1–8,

postpartum). With weekly testing, for example, a rise in apparent prevalence could be a signal to act.

When testing an individual cow that is suspected of subclinical ketosis, a positive urine test has little additional diagnostic value, due to the low specificity of the test. However, with a negative urine test, the blood BHB concentration will almost certainly be below 1.4 mmol/L. Positive and negative results of a milk test would have better additional diagnostic value, due to the high specificity and relatively high sensitivity of the test.

With knowledge of the sensitivity and specificity of the milk and urine tests at different thresholds for BHB, one can use them to monitor subclinical ketosis in a herd. However, milk testing is to be preferred, because of the easy obtainability of milk combined with the overall better test characteristics.

Acknowledgments

Pharmaceutical recipe of sodium nitroprusside powder was made available through Dr. R. Witkamp at the pharmacy of the School of Veterinary Medicine, Utrecht.

CvJ

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CORRECTIONS

Serologic evidence of porcine reproductive and respiratory syndrome (PRRS)—*Can Vet J* 1994; 35: 53

Alfonso Lopez, Carmencita Yason, Shelly Burton, Ed MacAulay

The last sentence in this article should have been referenced to number (4), not (3) as printed. The text should have been printed as follows:

"These lesions were morphologically different to those found in proliferative necrotizing pneumonia first described in Québec (4)."

Also, the name of one author of this article was inadvertently omitted. The correct text follows:

Alfonso Lopez, Carmencita Yason, Shelly Burton, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3; Ed MacAulay, Montague Veterinary Clinic, Montague, Prince Edward Island C0A 1R0.

Uterine prolapse in a camel—*Can Vet J* 1993; 34: 445

R.O. Ramadan, M.M. Al Eknah, A.M. Hafez

The name of one author of this article was inadvertently omitted. Dr. M.M. Al Eknah of King Faisal University worked with his colleagues as an author of this text.

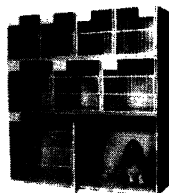
The editors regret the errors and offer sincere apologies to the authors mentioned above.

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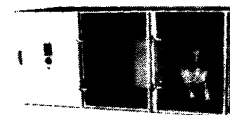
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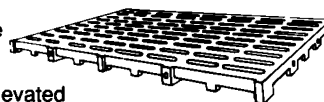
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